Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

Emergence, evolution, and vaccine production approaches of SARS-CoV-2 virus: Benefits of getting vaccinated and common questions

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ABSTRACT

The emergence of coronavirus disease 2019 (COVID-19) pandemic in Wuhan city, China at the end of 2019 made it urgent to identify the origin of the causal pathogen and its molecular evolution, to appropriately design an effective vaccine. This study analyzes the evolutionary background of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or SARS-2) in accordance with its close relative SARS-CoV (SARS-1), which was emerged in 2002. A comparative genomic and proteomic study was conducted on SARS-2, SARS-1, and Middle East respiratory syndrome coronavirus (MERS), which was emerged in 2012. In silico analysis inferred the genetic variability among the tested viruses. The SARS-1 genome harbored 11 genes encoding 12 proteins, while SARS-2 genome contained only 10 genes encoding for 10 proteins. MERS genome contained 11 genes encoding 11 proteins. The analysis also revealed a slight variation in the whole genome size of SARS-2 comparing to its siblings resulting from sequential insertions and deletions (indels) throughout the viral genome particularly ORF1AB, spike, ORF10 and ORF8. The effective indels were observed in the gene encoding the spike protein that is responsible for viral attachment to the angiotensin-converting enzyme 2 (ACE2) cell receptor and initiating infection. These

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SARS-CoV-1, Severe Acute Respiratory Syndrome Coronavirus 1; COVID-19, Corona Virus Disease 2019; ACE2, Angiotensin Converting Enzyme 2; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; WHO, World Health Organization; BLAST, Basic Local Alignment Search Tool; UTR, Untranslated region; CDC, Centers of Disease Control; NCBI, National Center for Biotechnology Information; NSP, nonstructural protein; ORF, Open Reading Frame; (S), Spike; (E), envelope; (M), membrane; (N), nucleocapsid; MDCK, Madin-Darby Canine Kidney; VOC, variants of concern; RBD, receptor binding domain; CD4, Helper T lymphocytes express cluster determinant 4; CD8, cytotoxic T cells express cluster determinant 8; PCR, polymerase chain reaction; NTD, N-Terminal Domain; mAbs, monoclonal antibodies; Del, Deletion; Ins, Insertion; PID, percentage identity; NJ, neighbor-joining.

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indels are responsible for the newly emerging COVID-19 variants αCoV, βCoV, γCoV and δCoV. Nowadays, few effective COVID-19 vaccines developed based on spike (S) glycoprotein were approved and become available worldwide. Currently available vaccines can relatively prevent the spread of COVID-19 and suppress the disease. The traditional (killed or attenuated virus vaccine and antibody-based vaccine) and innovated vaccine production technologies (RNA- and DNA-based vaccines and viral vectors) are summarized in this review. We finally highlight the most common questions related to COVID-19 disease and the benefits of getting vaccinated.

Contents

1. Introduction

The emergence of the pathogenic and highly contagious coronavirus disease 2019 (COVID-19) in China by the end of 2019 (Andersen et al., 2020), has threatened the world and raised the need to identify the origin and evolution of this virus. The causal agent of such disease referred as SARS-COV-2 with high pathogenicity and transmissibility among humans and animals. SARS-CoV-2, a member of the subfamily *Coronavirinae*, family *Coronavirdiae*, order *Nidovirales*, has a single stranded RNA genome of about 29.9 kb in size (Woo et al., 2010; Attia et al., 2021). Members of coronaviruses were sorted out into four subgenera referred as *Alph-acoronavirus* (*Alph*CoV), *Beta-coronavirus* (BetaCoV),-*Gamma-coronavirus* (GammaCoV) and *Delta-coronavirus* (DeltaCoV) (Chan et al., 2013). Evolution analyses among viral genome sequences inferred that human, bats and rodent are gene sources for *Alpha-* and β-*coronavirus*, while avian and other animal species are hosts for most *Delta-* and γ-*coronavirus*. The genus β-*coronavirus*, which includes the deadliest members that caused epidemics, contained four genetically distinct lineages termed A, B, C and D. Coronavirus members are delineated by genome organization and nucleotide sequence identity of the accessory ORFs (Chan et al., 2020). The most common pandemic-causing viruses, SARS-CoV (SARS-1) and SARS-CoV-2 (SARS-2), belong to lineage B (Stout et al., 2020)

COVID-19 disease causes unusual viral pneumonia including fever, cough, chest discomfort and inflammatory cell infiltration in multiple organs (Gralinski and Menachery, 2020; Xiao et al., 2020; Zhu et al., 2020). The SARS-2 virus can multiply in humans and few animal species including bats and pangolin. The horseshoe

bats are among the important natural hosts for AlphCoV and BetaCoV (Hu et al., 2021). The SARS-2 shares 96.2 and 93.3% nucleotide identity to its closest relatives RaTG13 and RmYN02 infecting the intermediate and the Malayan horseshoe bats, respectively (Zhou et al., 2020), which supports the hypothesis that such virus likely originated from bats.

The Virulence of SARS-2 virus infections in patients range itself from mild to severe respiratory failure. The coronaviruses SARS-1, MERS and SARS-2were reported as human pathogens. The virus enters through the epithelial cells lining the respiratory tract by interacting via its spike (S) glycoprotein with the cellular receptor ACE2 (angiotensin-converting enzyme 2) in presence of the cellular transmembrane serine protease TMPRSS2 (Wan et al., 2020; Wrapp et al., 2020). The viral S protein is made of two subunits termed S1 and S2 connected by a receptor- binding domain (RBD). The S1 subunit entails the surface unit that binds to ACE2, while S2 subunit drives the virion fusion to the cellular membrane. The evolutionary changes in viral genome via nucleotide insertions and/or deletions occurred as a normal evolutionary event during virus to produce viral variants with superior capabilities in virulence and fitness (Panzera et al., 2021; Wang et al., 2021b). Mutations within the viral S protein are of a great concern as they significantly affect virus transmissibility and evading immunity. Consequently, evolution of the viral genome and its encoded proteome essentially require the development of new vaccines. The knowledge of genetic variability among SARS-1 and SARS-2 genomes and proteomes through their evolutionary history enables us to develop proper vaccines and prevent the possible outbreak coming due to the new virus variants in the near future. These genetic variations may explain the first proposed theory for SARS-2 emergence and outbreak. Two possible hypotheses were proposed to interpret the SARS-2 outbreak (Kaina, 2021). The first one suggested the emergence of SARS-2 virus through natural selection in a natural ecological niche, and the second one suggested the development of a viral infectious clone mimicking coronaviruses with higher capabilities at the lab. Studying the genetic variations among coronavirus genomes and proteomes using comparative bioinformatics may shed the light on the evolutionary events that had been occurred before the emergence of SARS-2 in 2019. In this review, the perspectives on the acquired properties among the SARS-2 and SARS-1 genomes are highlighted. This result will expand our knowledge of the new variants of fast-base evolving viruses and help the scientific community to conclude the possible hypotheses leading to the virus emergence either by natural selection or by laboratory genetic manipulation (Barh et al., 2020). MERS genome was also considered in the current study to reveal any evolutionary relationship with SARS-2, since both can withstand a high temperature environment.

The common symptoms associated with SARS-2 infections are fever and pain of body chills, which may develop into severe pneumonia and death (Huang et al., 2020; Mehta et al., 2020). MERS symptoms are also including fever, cough, and shortness of breath, and infection may also lead to pneumonia (Gralinski and Menachery, 2020; Zhu et al., 2020). Currently, we already have several types of effective vaccines worldwide. This review summarized the evolutionary changes among SARS-2 (2019) and SARS-1 (2002), COVID-19 diagnosis, treatment, and approaches of vaccine production.

2. Source of genomic sequences

SARS-2, SARS-1 and MERS genomic sequences were obtained from GenBank database under the accession numbers MN908947.3, AY278489.2, and NC_019843.3, respectively. Genome was annotated using the NCBI tools, and the gene/protein sizes were predicted for SARS-1, SARS-2 and MERS genomes.

3. SARS-2 and SARS-1 protein alignment

The sequences of deduced protein of all viral genes of SARS-1and SARS-2were aligned using Clustal Omega online tool software (Madeira et al., 2019) to determine the variable and conserved regions. The amino acid sequences were further used to determining potential functions, structures and evolutionary relationship between the two viruses. The presence of nucleotide insertion/deletion mutations were also studied using multiple sequence alignment tools. Comparing the number of genes and their deduced proteins were performed to understand the virus strength, virulence, and the behavior of the virus.

4. Characteristics of SARS-1, SARS-2 and MERS genomes

The genome organization of such newly emerged virus is similar to BetaCOV (lineage B) genome organization. The viral genome encompasses approximately ten ORFs flanked by 5′- and 3′-untranslatable regions. The 5′ terminus of the viral genome and subgenomic RNAs are capped and the 3′ terminus is poly adenylated. The functional ORFs within the viral genome are arranged from 5′ to 3′ in the following order: replicase (ORF1a/1b), spike glycoprotein (S), glycoprotein envelope (E), membrane glycoprotein (M) and nucleocapsid (N) (Table 1). Five to nine more putative accessory proteins are placed among the structural genes (Chan et al., 2020). The number of accessory proteins encoded by the 3′subgenomic RNAs is variable among the virus species. The virus expresses its nonstructural proteins through primary translation and polyprotein processing, and the structural and accessory proteins *via* subgenomic RNAs. The viral replicase is located at the 5′-sideand covers more than two thirds of the viral genome. The replicase coding sequence ORF1ab encodes for the polyproteins pp1a and pp1b, with the latter getting expressed *via* a ribosomal −1 frameshift. The polyproteins get eventually processed into 16 distinct nonstructural proteins (nsp1 to nsp16) that are required for transcription and replication (Table 2). Proteolytic cleavage is directed by two cysteine proteases namely nsp3 (papain-like protease) and nsp5 (chymotrypsin-like protease) (Chan et al., 2020). The roles of the four structural proteins of coronaviruses are simply outlined in Fig. 1. The accessory proteins are not important for the replication of the virus but seem to have a role in pathogenecity.

The bioinformatic analysis of SARS-1 genome (29.757 kb) proved the existence of11 different genes encoding for 14proteins flanked by 248 nt 5′-untranslated region (5′-UTR) and 1317 nt 3′-untranslated region (3′-UTR) regions. The genome of SARS-2 (29.903 kb) published in March of 2020 on the GenBank database revealed the presence of 10 ORFs encoding for 10 proteins arranged in a similar organization and flanked by 265 nt 5′-UTR and 228 nt 3′-UTR regions. The MERS genome (30.119 kb) consists of 11 genes encoding for 11 proteins flanked by 278 nt 5′-UTR and 1018 nt 3′-UTR regions, as presented in Table 1 and Fig. 1. The novel betacoronavirus SARS-2 shares about 79% nucleotide identity with SARS-1 and 50% with MERS (Lu et al., 2020).

Phylogenetic analysis enabling the estimating of the evolutionary relationships among organisms using the sequence of a common gene or protein (Hassanin et al., 2020; Raza et al., 2021). The phylogeny analysis for the complete viral genome showed that SARS-2 has clustered with SARS-1 and other SARS-related coronaviruses found in horseshoe bats and pangolin, placing it in the subgenus Sarbecovirus of the genus *Beta-coronavirus* (Fig. 2). The same phylogeny has placed MERS-CoV in the subgenus Merbecovirus within the same genus. The number of accessory proteins

Table 1
Comparative genomic and proteomic analyses of SARS-1, SARS-2 and MERS viruses. Gene size is measured in nucleotides (nt) and protein size is estimated in amino acids (aa).

Gene no.	**SARS-1**(AY278489.2)				**SARS-2** (MN908947.3)				**MERS**(NC_019843.3)			
	Gene name	Gene location	Gene length	Protein size	Gene name	Genomic location	Gene length	Protein size	Gene name	Gene location	Gene length	Protein size
	5′UTR	1–248	248		**5′UTR**	1–265	265		**5′UTR**	1–278	278	
1	**ORF1a**	249–13397	13,148	4382	**Orf1a**	266–13483	13,217	4405	**orf1a**	279–13454	13,175	4391
2	**ORF1ab**	249–21469	21,220	7073	**Orf1ab**	266–21555	21,289	7096	**orf1ab**	279–21514	21,235	7078
3	**S**	21476–25243	3767	1255	**S**	21563–25384	3821	1273	**S**	21456–25517	4061	1353
4	**ORF3a**	25252–26076	824	274	**ORF3a**	25393–26220	827	275	**orf3**	25532–25843	311	103
5	**ORF3b**	25673–26137	500	154					**orf4a**	25852–26181	329	109
6	**E**	26101–26331	230	76	**E**	26245–26472	227	75	**orf4b**	26093.0.26833	740	246
7	**M**	26382–27047	665	221	**M**	26523–27191	668	222	**orf5**	26840–27514	674	224
8	**ORF6**	27058–27249	191	63	**ORF6**	27202–27387	185	61	**E**	27590–27838	248	82
9	**ORF7a**	27257–27625	368	122	**ORF7a**	27394–27759	365	121	**M**	27853–28512	659	219
10	**ORF8**	27763–28131	368	122	**ORF8**	27894–28259	365	121	**N**	28566–29807	1241	413
11	**N**	28133–29401	1268	422	**N**	28274–29533	1259	419	**orf8b**	28762–29100	338	112
12	**ORF10**	28143–28439	296	98	**ORF10**	29558–29674	116	38				
	3′UTR	28440–29757	1317		**3′UTR**	29675–29903	228		**3′UTR**	29101–30119	1018	
Genome size:			29,757 nt				29,903 nt				30,119 nt	

Genomic data were obtained from the NCBI GenBank database and analyzed using the proper bioinformatics tools.

Table 2
Positions and potential roles of SARS-2 nonstructural proteins (nsp) in ORF1ab as predicted by bioinformatics tools.

NSPs	Protein IDs for SARS-2	amino acid (aa) position	aa identity % with		Putativerole
			SARS-1	MERS	
Nsp1	YP-009725297.1	M1 – G180	84	21	Suppress antiviral host response
Nsp2	YP-009725298.1	A181 – G818	68	22	proofreading viral replication
Nsp3	YP-009725299.1	A819 – G2763	76	33	putative papain-like-protease domain
Nsp4	YP-009725300.1	K2764 – Q3263	80	40	complex with nsp3 and 6: contains transmembrane domain 2 (TM2)
Nsp5	YP-009725301.1	S3264 – Q3569	96	51	3CL-pro domain and main protease
Nsp6	YP-009725302.1	S3570 – Q3859	88	35	complex with nsp3 and 4: putative transmembrane domain
Nsp7	YP-009725303.1	S3860 – Q3942	99	56	complex with nsp8: RNA-dependent RNA polymerase
Nsp8	YP-009725304.1	A3943 – Q4140	97	53	complex with nsp7: Multimeric RNA polymerase; replicase
Nsp9	YP-009725305.1	N4141 – Q4253	97	54	ssRNA binding activity
Nsp10	YP-009725306.1	A4254 – Q4392	97	59	complex with nsp14: proofreading, contains two zincbinding motifs
Nsp11	YP-009725312.1	S4393 – V4405	85	34	short peptide at the end of orf1a
Nsp12	YP-009725307.1	S4393 – Q5324	96	72	RNA-dependent RNA polymerase
Nsp13	YP-009725308.1	A5325 – Q5925	100	72	Helicase, Zinc-binding domain
Nsp14	YP-009725309.1	A5926 – Q6452	95	63	ExoN: 3′–5′ exonuclease
Nsp15	YP-009725310.1	S6453 – Q6798	89	52	EndoU: poly(U)-specific endoribonuclease
Nsp16	YP-009725311.1	S6799 – N7096	93	66	2′-O-ribose methyltransferase
S	QHD43416.1	1 – 1273	76	33	Spike protein
orf3a	QHD43417.1	1 – 275	72	21	Accessory protein
E	QHD43418.1	1 – 75	95	37	Envelop protein
M	QHD43419.1	1 – 222	91	40	Membrane protein
orf6	QHD43420.1	1 – 61	69	–	Accessory protein
orf7a	QHD43421.1	1 – 121	85	–	Accessory protein
orf8	QHD43422.1	1 – 121	40	30	Accessory protein
N	QHD43423.2	1 –419	94	49	Nucleocapsid phosphoprotein
Orf10	QHI42199.1	1 – 38	–	–	Accessory protein

present on the 3′-third of the viral genome is among the distinctive features of coronavirus lineages (Fig. 3 and Tables 3 and 4).

The genetic analysis displayed in Table 1 refers to the presence of five common genes termed ORF1ab, S, E, M, and N within the three investigated viral genomes. The SARS-2 and SARS-1were closely related to each other as they shared the same open reading frames (ORFs) (ORF1ab, S, ORF3a, E, M, ORF6, ORF7a, ORF8, N and ORF10) throughout their genome. Most of the nonstructural polyprotein units located in the replicase polyprotein (ORF1ab) share more than 85% amino acid identity among SARS-1 and SARS-2 (Chan et al., 2020). The proteins encoded by both viruses have a similar length. About 90% of amino acid identity was

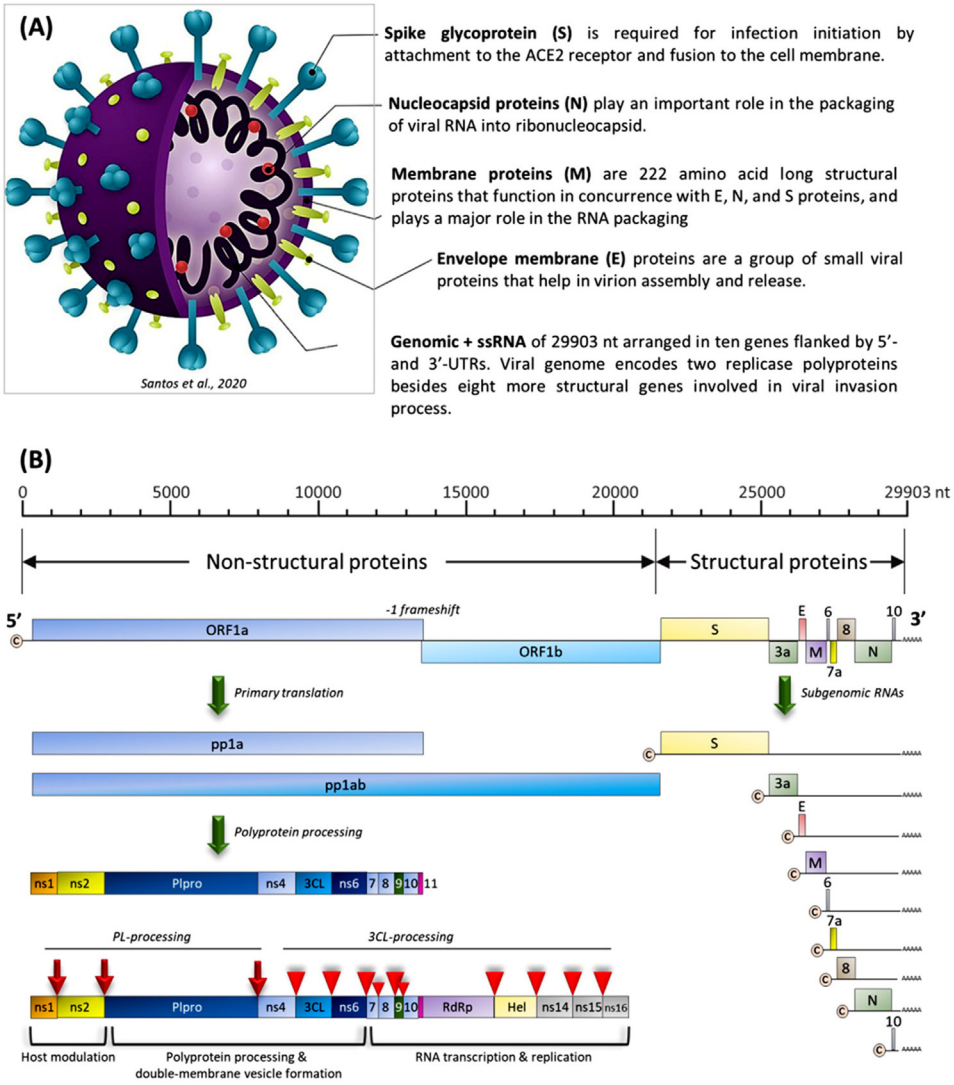

Fig. 1. Schematic representation of the SARS-2 virion and RNA genome. A) Structure of SARS-2 virion particle, and the function of its structural proteins. **B)** Genome organization gene expression strategies of SARS-2. The 5′-proximal two-thirds of the genome contains two overlapping open reading frames (ORF1a and ORF1b) that encode two long polyprotein precursors, namely pp1a and pp1ab, respectively.

observed between the four structural proteins of both viruses, with an exception for the S protein, which exhibited a higher level of divergence (Table 2) (Lu et al., 2020; Zhou et al., 2020). The spike protein of SARS-2 full length (1273 amino acids) was longer than that of SARS-CoV (1255 a.a.), but much shorter than that of MERS-1 (1353 a.a.). The SARS-2 S protein shares 77% amino acid sequence similarity with SARS-1, 75–97% with horseshoe bats coronaviruses, and 90–92% with pangolin coronaviruses (Zhou et al., 2020). Within the spike protein of SARS-2, the receptor-binding domain (RBD) shares only 73% amino acid similarity with SARS-1. In addition, the four amino acid insertion (PRRA) between the two protein subunits of the S protein in SARS-2 distinguishes it from other members within the same lineage. This insertion enables effective cleavage by several types of proteases, which is thought to enable higher virus transmissibility comparing to the SARS-1 (Andersen et al., 2020). Another difference is the ORF8 in SARS-2, which encodes a protein that shows only 40% amino acid identity to ORF8 of the SARS-1. Such novel accessory gene lacks the motif responsible for triggering the intracellular stress pathways(Chan et al., 2020). On the other hand, SARS-2 shared only five genes with MERS. The amino acid composition of RBD domain within the S protein was also different from that in SARS-2 (Naqvi et al., 2020). Furthermore, the analysis concluded that the order of genes was different between MERS and SARS genomes, which indicate the genetic variation between those two distantly related lineages. The upcoming research will focus on the closely related virus members SARS-1, SARS-2.

5. Molecular evolution of coronavirus

The evolutionary origin of viruses has been considered central dogma to understand the origin of the viruses and their evolutionary relationships (Bandea, 2009; Swelum et al., 2020). Comparing the SARS-2 virus with the MERS shows that SARS-2 was distantly related to the MERS. The phylogeny tree based on the complete genome showed that SARS-2 and SARS-1were clustered together (Wu et al., 2020). At the complete genome level, the SARS-2 shares an 79% sequence identity with the SARS-1 but only about 50% sequence identity with the MERS (Lu et al., 2020).

The analysis of SARS-2 and SARS-1 genomes revealed that the genomic ORF1ab of SARS-2 conceived two insertions at 72 and 6 nucleotide positions, which alter the amino acids 993 and 1211, respectively (Fig. 4). These changes may be corresponding to the cleavages incurred at the N-terminus essentially required for virus

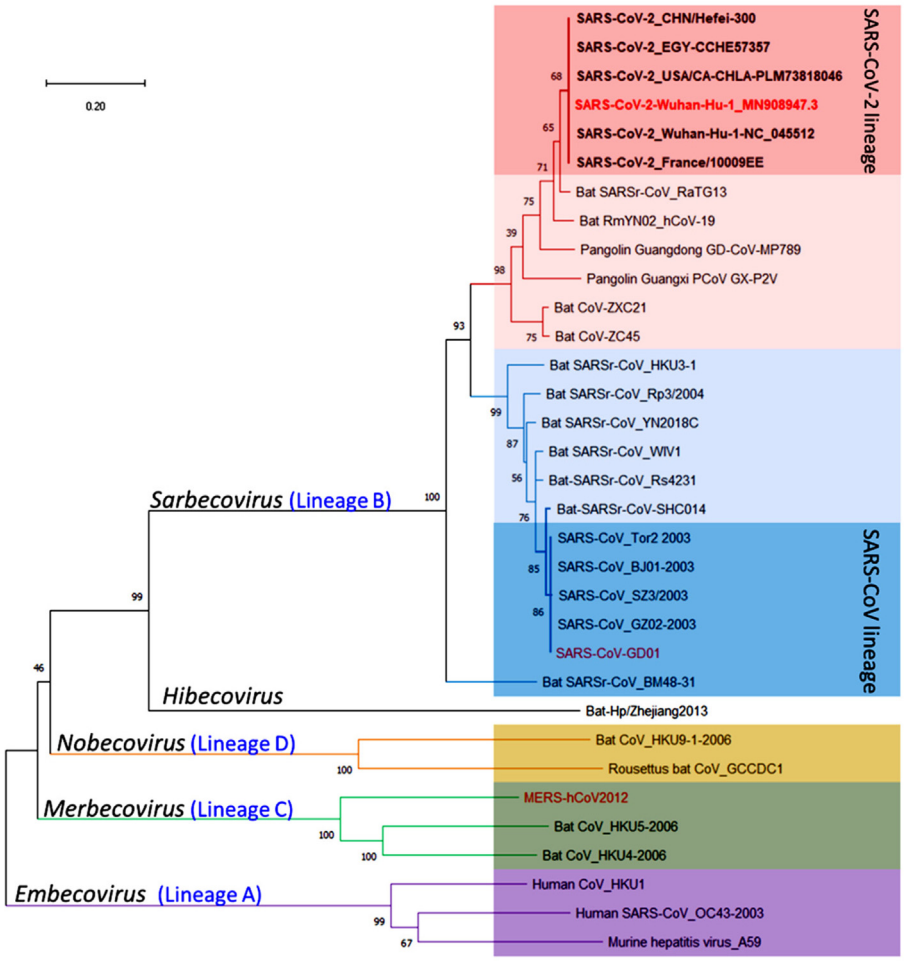

Fig.2. Phylogenetic analysis revealing the descendant subspecies of genus *Betacoronavirus*. Full-length genomic RNA sequences representing members from every subspecies were included along with the genomes of SARS-CoV, SARS-CoV-2 and MERS-CoV (in red). The phylogenetic trees were calculated from distance mattresses determined from percentage identity (PID) using neighbor-joining (NJ) algorithm. Genomic sequences were retracted from the GenBank and GISAID database under accession numbers shown in. Multiple sequence alignment was done using Clustal Omega online tool(Madeira et al., 2019), data were curated manually using Jalview2(Waterhouse et al., 2009), and phylogeny was done using MEGA X software with bootstrap values calculated from 1000 replicates(Kumar et al., 2018). Subspecies and corresponding lineages are shown on branches.

replication (Wu et al., 2020). The analysis also revealed that SAR-2 genome had three deletions (9 nt) corresponding to three amino acids at positions, 823, 933, and 1539 of the orf1ab polyprotein (Table 5 and Fig. 1A). In the same context, spike protein gene has two deletions (12 nt) that corresponding to amino acids at positions 21 and 31 and five insertions (66 nt) altered the amino acids at positions 69, 149, 247,483 and 679 (Table 5 and Fig. 5B). Considerable genetic variation was observed in ORF8-encodedprotein, which is responsible for the host-virus interaction. Three insertions (15 nt) altered the amino acids at positions 15, 61 and 71 and two deletions (18 nt) altered the amino acid positions 85 and 122 (Table 5 and Fig. 7B). The ORF10 sequence also showed significant alterations represented by three deletions (181 nt) caused alteration in the amino acids at positions 1, 5, and 38 and one insertion (3 nt) altered the amino acid at position 26 (Table 5 and Fig. 7D). Based on the data in Table 5, the genes that showed small deletions and insertions were ORF3a and M genes, which exhibited a 3 nt insertion in the genomic sequence and its corresponding amino acid positions 241 and 1, respectively (Table 5 and Fig. 6A and C). The E gene had a deletion of 3 nt corresponding to the amino acid at position 70 (Table 5 and Fig. 6B). The ORF6 had one deletion of 6 nt caused the alteration of amino acid at position 62 (Table 5 and Fig. 6D), and ORF7a had a deletion of 3 nt altered the amino acid at position 95 (Table 5 and Fig. 7A), while the N gene has two deletions of 9 nt amino acids at positions 8 and 420 (Table 5 and Fig. 7C).

Molecular evolution rate for various viruses species ranged from $0.46x10^{-4}$ nucleotide substitution/site/year for *Sudan ebolavirus* to $8.21x10^{-4}$ for *Reston ebolavirus* (Carroll et al., 2013). Based

Lineage:	Type species:	Host:	3'- genome organization
A	HCoV_HKU1	human	
B	SARS-CoV-2	human	
	SARS-CoV	human	
C	MERS-CoV	human	
	Bat CoV_HKU4	bat	
D	Vat CoV_HKU9	bat	

Fig. 3. Taxonomic lineages of genus *Betacoronavirus*. Four genetically distinct lineages are designated A, B, C and D. representative example for each lineage is shown. The variable accessory ORFs at the 3′-third of the viral genomes are shown. Boxes colors match those of each corresponding subspecies shown in (Fig. 2). Open boxes represent consensus viral ORFs.

Table 3
List of coronavirus isolates used to study the phylogenetic relationship. Accession numbers for the isolates studied in the current research are shown in bold and underlined font. The year of discovery and identification is also shown.

	Coronavirus isolate:	GenBank ID:	Year
1	SARS-CoV GZ02	AY390556	2003
2	SARS-CoV SZ3	AY304486.1	2003
3	SARS-CoV Tor2	AY274119	2003
4	SARS-CoV BJ01	AY278488	2003
5	**SARS-CoV GD01**	AY278489.2	2003
6	Bat SARSr-CoV Rs4231	KY417146	2016
7	Bat SARSr-CoV SHC014	MT308984.1	2013
8	Bat SARSr-CoV WIV1	KF367457	2013
9	Bat SARSr-CoV YN2018C	MK211377	2018
10	Bat SARSr-CoV Rp3	DQ071615.1	2004
11	Bat SARSr-CoV HKU3-1	DQ022305	2004
12	Bat SARSr-CoV BM48-31	NC014470	2008
13	Pangolin coronavirus Guangxi	MT072864	2020
14	Bat coronavirus ZXC21	MG772934	2018
15	Bat coronavirus ZC45	MG772933	2018
16	Pangolin coronavirus Guangdong	MT121216	2019
17	Bat coronavirus RmYN02	EPI_ISL_412977	2020
18	Bat coronavirus RaTG13	MN996532	2013
19	**SARS-CoV-2_Wuhan-Hu-1**	MN908947.3	2020
20	SARS-CoV-2_Egy_CCHE57357	MZ380257.1	2021
21	SARS-CoV-2_ USA/CA-CHLA-PLM73818046	MZ722702.1	2020
22	SARS-CoV-2_ /France/10009EE	MT470142.1	2020
23	SARS-CoV-2_ CHN/Hefei-300	MZ824630.1	2020
24	Bat Hp-betacoronavirus Zhejiang2013	KF636752.1	2013
25	Rousettus bat coronavirus HKU9	EF065513	2006
26	Rousettus bat coronavirus GCCDC1	MT350598.1	2016
27	Pipistrellus bat coronavirus HKU5	NC009020	2006
28	Tylonycteris bat coronavirus HKU4	NC009019	2006
29	**MERS-CoV_EMC**	NC_019843.3	2012
30	Human coronavirus HKU1	NC006577	2005
31	Murine hepatitis virus_A59	NC_048217.1	2005
32	Human coronavirus OC43	AY391777	2003

on the previously mentioned data, the molecular evolution rate for SARS-2 was $8.5x10^{-4}$ nucleotide substitutions/site/year. The new extra amino acids added to the encoded protein of SARS-2particularly in S glycoprotein maybe the reason for the new virulence and transmissibility traits of this virus including the capability to bind to the receptor of human epithelial cells. The novel features of SARS-2 genome may precise the structural changes of its genome that responsible for the high transmissibility of this virus in humans around the world. Although the analysis refuses the manipulation of SARS-COV-2 virus, more scientific investigation is required on other viral isolates to determine the real origin of the virus.

6. Diagnostics for COVID-19

Molecular approaches such as qRT-PCR analyses using specific primers and next-generation sequencing (NGS) are precise molecular techniques to identify the viral RNA sequence in patients. The detection of SARS-2 sequence targets the conserved genes in the genome of the virus N and EORFs (Yu et al., 2020). The samples for COVID-19 examination can be sputum, blood, nasopharyngeal swabs, lower respiratory tract secretions and feces. Yang et al. (2020) examined the nasal swab, throat swab, sputum, and bronchoalveolar fluid in patients to assess the molecular diagnostic assays approved by the FDA and found that highest diagnostic accuracy had scored on sputum and nasal swabs samples, respectively. Another investigation for detecting SARS-2 nucleic acid sequences in saliva indicated the presence of the viral sequence in 92% of COVID-19 patient's saliva (To et al., 2020). Despite the fact that qRT-PCR is highly sensitive, the accuracy of such test could be easily muddled by several factors such as the sample type, the methods of sample preservation, the time length of viral RNA extraction and the efficiency reagents used for extraction. Therefore, a reliable low cost, efficient and one-step serology-based alternative is urgently needed.

7. Clinical features of coronavirus disease

The symptoms of coronavirus disease initially resemble pneumonia caused by other viruses and bacteria, with a different level of severity. The incubation period of SARS-2 is usually between three to seven days, according to Center of Disease Control (CDC), and sometimes extends up for two weeks. In some cases, COVID-19-infected patients may remain symptomless. The initial symptoms appear due to COVID-19 infection include fever, cough, and shortness of breath are among (Fig. 8). Eventually, the patient's health worsen and more symptoms appear as chills, muscle pains, sore throat, and loss of taste and smell were later included to the list (Control and Prevention, 2020). Some patients may suffer headache and myalgia, while others may have gastrointestinal problems and diarrhea. The severe symptoms usually are hard breathing and dyspnea in the beginning of the second week after the start of symptoms, and the symptoms may develop to acute respiratory distress syndrome, septic shock, metabolic acidosis, and coagulopathy. Interestingly, some severely ill persons initially have mild symptoms like low-grade of fever and mild cough, but they rapidly deteriorate (Organization, 2020). Among COVID-19 patients, about 80% show intermediate illness, while only 14% show severe illness, and 5% require intensive care or mechanical ventilation assistance(Novel, 2020). The risk of severe illness increased with old people and patients of diabetes, chronic obstructive pulmonary diseases, hypertension, and heart diseases.

Table 4
The distance identity mattresses used to draw the phylogenetic relationship among SARS coronaviruses. Percentages of identity were calculated by Clustal 12.1.

	1	2	3	4	5	6	7	8	9	10	11	12	3	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1	100																																
2	72.4	100																															
3	72.5	72.4	100																														
4	50.3	50.4	49.4	100																													
5	52.0	52.8	50.7	72.4	100																												
6	51.1	51.5	50.2	70.0	70.2	100																											
7	51.5	52.4	50.5	50.6	52.2	51.1	100																										
8	49.4	49.9	49.3	50.3	50.8	50.4	66.5	100																									
9	49.5	50.1	49.2	51.1	51.7	51.7	52.0	51.0	100																								
10	50.9	51.6	50.7	51.8	52.9	52.5	52.7	51.6	57.0	100																							
11	50.7	51.1	50.2	52.0	52.7	52.6	52.0	51.3	56.9	78.5	100																						
12	50.7	51.0	50.1	52.0	52.6	52.5	52.0	51.3	56.7	78.6	87.9	100																					
13	50.8	51.0	50.1	52.0	52.7	52.6	52.1	51.3	56.8	78.7	87.9	98.5	100																				
14	50.8	51.0	50.1	52.0	52.7	52.6	52.1	51.3	56.8	78.7	87.9	98.4	100	100																			
15	50.8	51.0	50.1	52.0	52.7	52.6	52.1	51.3	56.7	78.7	87.9	98.4	99.8	99.8	100																		
16	50.8	51.0	50.1	52.0	52.7	52.6	52.1	51.3	56.8	78.7	87.9	98.4	99.9	99.9	99.9	100																	
17	50.8	50.9	50.1	52.0	52.7	52.6	52.1	51.3	56.7	78.7	87.8	98.4	99.8	99.9	99.8	99.9	100																
18	50.7	50.8	50.0	51.9	52.6	52.6	52.1	51.5	56.8	78.8	88.3	95.6	95.7	95.7	95.7	95.7	95.7	100															
19	50.8	51.1	50.2	52.0	52.7	52.7	52.1	51.5	57.0	78.7	88.3	96.4	95.6	95.6	95.5	95.6	95.5	96.8	100														
20	50.8	51.0	50.1	51.9	52.6	52.5	52.1	51.4	56.9	78.8	89.7	93.9	93.9	93.9	93.8	93.8	93.8	95.5	95.6	100													
21	50.9	51.0	50.2	52.0	52.8	52.6	52.0	51.4	57.0	78.7	89.7	92.5	92.5	92.5	92.5	92.5	92.4	93.4	93.5	95.3	100												
22	51.2	52.2	50.4	52.3	53.4	52.8	52.5	51.6	57.2	76.1	78.7	78.9	78.8	78.8	78.9	78.9	78.8	79.1	79.2	79.1	79.0	100											
23	51.4	52.4	50.8	52.2	53.4	52.8	52.9	51.7	57.4	76.2	79.1	79.3	79.4	79.4	79.4	79.5	79.4	79.1	o	79.3	79.3	83.5	100										
24	51.5	52.5	50.7	52.2	53.5	52.9	52.8	51.6	57.5	76.4	79.3	79.3	79.3	79.3	79.3	79.3	79.3	79.5	79.5	79.6	79.4	85.2	93.2	100									
25	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.3	79.5	79.5	79.5	79.5	79.5	79.4	79.6	79.6	79.6	79.5	85.4	93.9	96.2	100								
26	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.4	79.5	79.5	79.5	79.5	79.5	79.5	79.6	79.7	79.6	79.5	85.4	93.9	96.2	100	100							
27	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.3	79.5	79.5	79.5	79.5	79.5	79.4	79.6	79.6	79.6	79.5	85.4	93.9	96.2	100	100	100						
28	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.4	79.5	79.5	79.5	79.5	79.5	79.5	79.6	79.6	79.6	79.5	85.4	93.9	96.2	100	100	100	100					
29	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.4	79.5	79.5	79.5	79.5	79.5	79.5	79.6	79.6	79.6	79.5	85.4	93.9	96.2	100	100	100	100	100				
30	51.4	52.6	50.9	52.4	53.6	52.8	53.0	51.8	57.5	76.5	79.4	79.5	79.5	79.5	79.5	79.5	79.5	79.6	79.6	79.6	79.5	85.4	93.9	96.2	100	100	100	100	100	100			
31	51.3	52.1	50.8	52.3	53.3	52.8	52.6	51.4	57.2	76.1	78.8	78.9	78.9	78.9	78.9	78.9	78.9	79.0	79.1	79.2	78.9	85.1	88.3	90.2	90.3	90.3	90.3	90.3	90.3	90.3	100		
32	51.2	52.1	50.4	52.1	53.3	52.6	52.8	51.5	57.3	76.8	82.3	81.0	81.0	81.0	81.0	81.0	80.9	81.1	81.2	81.5	81.5	83.3	87.3	88.0	88.0	88.0	88.0	88.0	88.0	88.0	87.1	100	
33	51.1	52.2	50.5	52.2	53.3	52.6	52.8	51.5	57.4	76.7	82.7	80.9	80.9	80.9	80.9	80.9	80.8	81.0	81.2	81.4	81.4	83.3	87.2	88.0	88.0	88.1	88.0	88.0	88.0	88.0	87.1	97.5	100

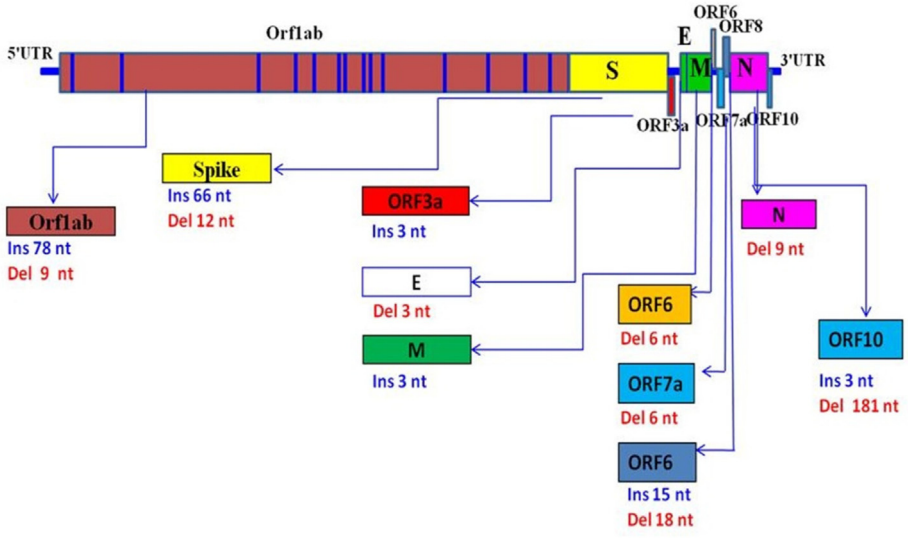

Fig. 4. Naturally occurring indels throughout SARS-CoV-2 genome. Boxes indicate the genomic size, number of inserted and deleted nucleotides of the viral genes. Del: Deletion, Ins: Insertion.

Table 5
Cumulative mutations within conserved ORFs throughout SARS-2 genome. Genetic variations were among SARS-1 and SARS-2 genomes are presented as number of nucleotide insertions, deletions and number of loci.

Genes	Insertions		Deletions		Indels
	no. of nucleotides	no. of loci	no. of nucleotides	no. of loci	total loci
Orf1ab	78	2	9	3	5
S	66	2	12	5	7
ORF3a	3	1	–	–	1
E	–	–	3	1	1
M	3	1	–	–	1
ORF6	–	–	6	1	1
ORF7a	–	–	3	1	1
ORF8	15	3	18	2	5
N	–	–	9	2	2
ORF10	3	1	181	3	4
Total	**168**	**10**	**241**	**18**	–

Importantly, according to the latest information, most COVID-19 patients recovered, while only 0.5 to 5% of patients will have severe/critical illness (Chen et al., 2020a).

8. Treatment of coronavirus disease

Due to the lack of efficient and specific treatments and the need to contain the epidemic, a new strategy must be sought to avoid the viral genomic and proteomic alterations, which enable viruses to escape the natural and developed immunity, a pathway may be potentially useful after receiving due research. This approach represents the basic proteins and peptides that can be natively available, e.g. lactoferrin or chemically designed by esterification which neutralizes the negatively charged carboxyl groups of the aspartyl glutamyl residues on protein molecules, transforming the protein net charge into positive (Sitohy et al., 2002). Cationic esterified proteins can interact with many microorganisms by virtue of their positive charge and their hydrophobic domains.

Different reports have confirmed this action against bacteria and fungi (Abdel-Shafi et al., 2016; El-Sayed et al., 2019). Esterified proteins were proven to *in vitro* interact with and complex DNA (Sitohy et al., 2002) were subsequently found to inhibit DNA amplification *in vitro* (Sitohy et al., 2001) and the replication of M13 bacteriophage and lactococcal bacteriophages (Sitohy et al., 2006). Human virues were found susceptible to esterified proteins (Chobert et al., 2007) as well as plant viruses (Abdelbacki et al., 2010). More relevantly, human Influenza virus A subtype H1N1 and human influenza virus A subtype H3N2 infected into MDCK cell lines were observed to be inhibited by methylated β-lactoglobulin. (Sitohy et al., 2010). A lethal Egyptian avian influenza A (H5N1) virus infected to MDCK cell lines was reported to be significantly inhibited by esterified whey proteins (Taha et al., 2010). Globally, these results suggest a wide-spectrum specificity of these chemically modified proteins against different virus and pathogenic bacteria nominating them as potential effective candidate in treating Covid-19.

Scientists worldwide did their best to develop effective treatments for COVID-19 as response to this serious worldwide pandemic. Several attempts were made to design new drug with known antiviral activities such as interferon. The control of the immuno-pathogenicity using immunomodulatory drugs to permit the lung a chance to recover was another avenue for COVID-19 therapy (ElBagoury et al., 2020). In addition, the use of passive immunotherapy using serum from coronavirus convalescents and stem cells for lung tissue regeneration has also been developed. Early attempts focused on determining existing drugs that might have antiviral effects.

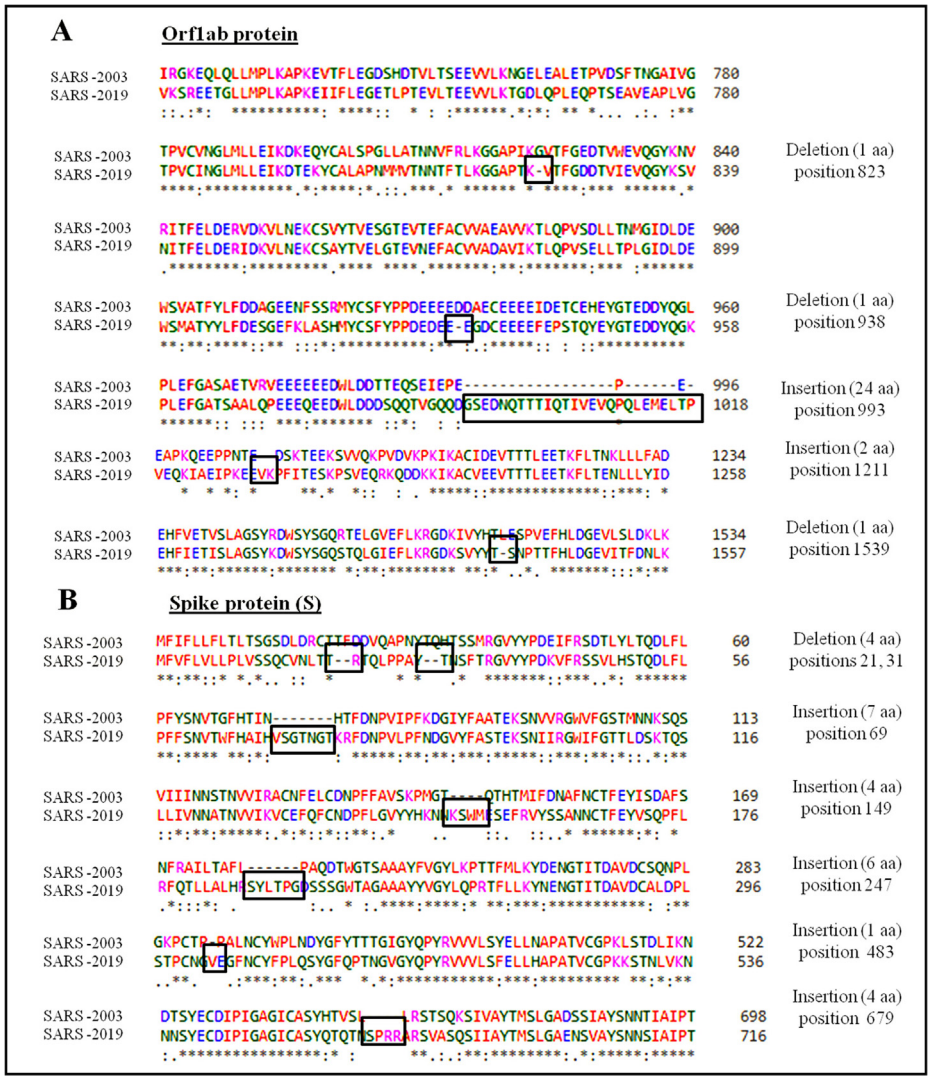

Fig. 5. CLUSTAL O (1.2.4) multiple proteins sequences alignment of SARS-CoV-2 (SARS-2) and SARS-COV (SARS-1) proteome. (A) Alignment of the variable regions of Orf1ab protein. (B) Alignment of the variable regions of Spike protein (S). Del: Deletion, Ins: Insertion.

Lots of studies on known drugs showed antiviral activities against COVID-19 including interferon, chloroquine, ribavirin, lopinavir/ritonavir. Protocols including those remedies were initially applied in China as the first affected country (Chen et al., 2020b; Huang et al., 2020; Xu et al., 2020). The old anti-malarial Chloroquine has high lipid solubility as well as its pH-dependent antiviral effects including coronaviruses. Hydroxychloroquine combined with azithromycin also showed a decreasing of virus load during treatment in France(Gautret et al., 2020). Another Chinese studies compared hydroxychloroquine alone versus standard care also provided a beneficial effects(Chen et al., 2020b). Conversely, some reports from Spain, United Kingdom, and USA suggesting the lack of benefits of hydroxychloroquine and azithromycin in curing coronavirus patients (Mitjà et al., 2020; Rosenberg et al., 2020).

9. Newly emerged SARS-2 variants

Variants of SARS-2 have been emerged and spread worldwide since the beginning of the coronavirus pandemic. Those variants were sorted into lineages based on their genetic identity. A group of viruses within a lineage are supposedly derived from a common ancestor.

Continuous sequencing of SARS-2 genome is mandatory to identify and track the newly evolved virus variants. Among the first emerged genetic mutations was the globally dominant D614G substitution, which enhances transmissibility and infectivity but without severe illness symptoms (Korber et al., 2020). A virus variant contains one or more substitution or deletion mutations within the S protein. Several virus variants were identified and characterized from human and animal samples such as those transmitted from farmed mink in Denmark, which had low transmissibility (Oreshkova et al., 2020). Various other variants of SARS-2 were characterized and designated as variants of concern (VOC) because of their ability to cause increased virulence, evade the neutralization by antibodies or reduce treatment or vaccination sensitivity. The continuously emerging of genetic variant surged the World Health Organization (WHO) established a classification system for determination of the emergence of SARS-2variants. The WHO also proposed using Greek alphabet, e.g., Alpha, Beta, and Gamma as a practical method to refer the viral variants. The characterized variants and their labels are outlined in Fig. 9 and the potential roles for their characterizing mutations are explained in Table 6 (Khateeb et al., 2021; Otto et al., 2021). Other variants of less importance such as kappa variant were not discussed.

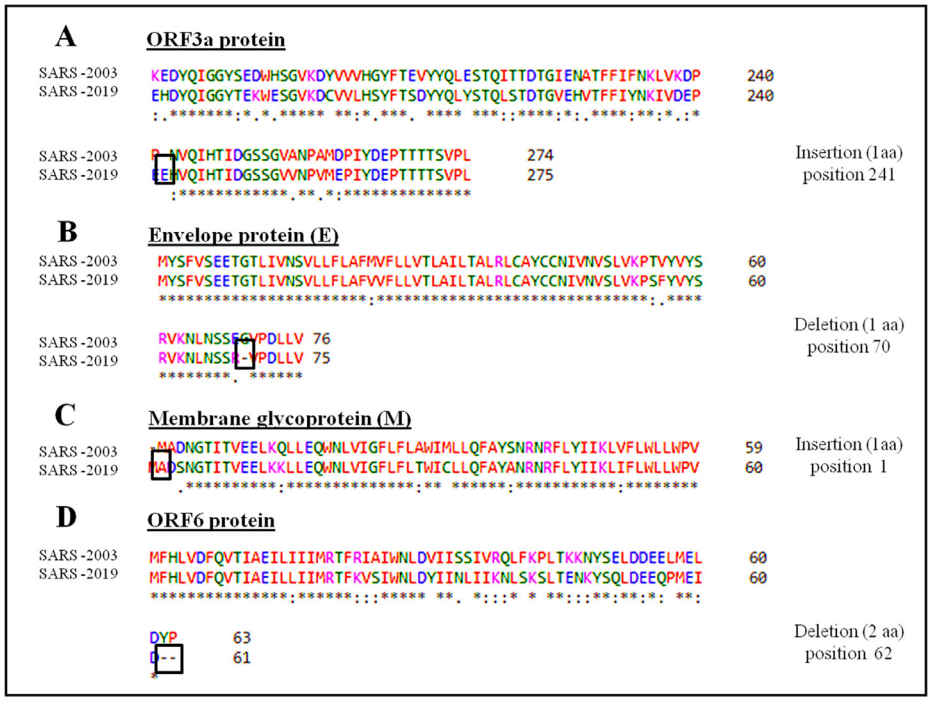

Fig. 6. CLUSTAL O (1.2.4) multiple proteins sequences alignment of SARS-CoV-2 (SARS-2) and SARS-COV (SARS-1) proteome. (A) Alignment of the variable regions of ORF3a protein. (B) Alignment of the variable regions of envelope protein (E). (C) Alignment of the variable regions of membrane glycoprotein (M). (D) Alignment of the variable regions of ORF6 protein. Del: Deletion, Ins: Insertion.

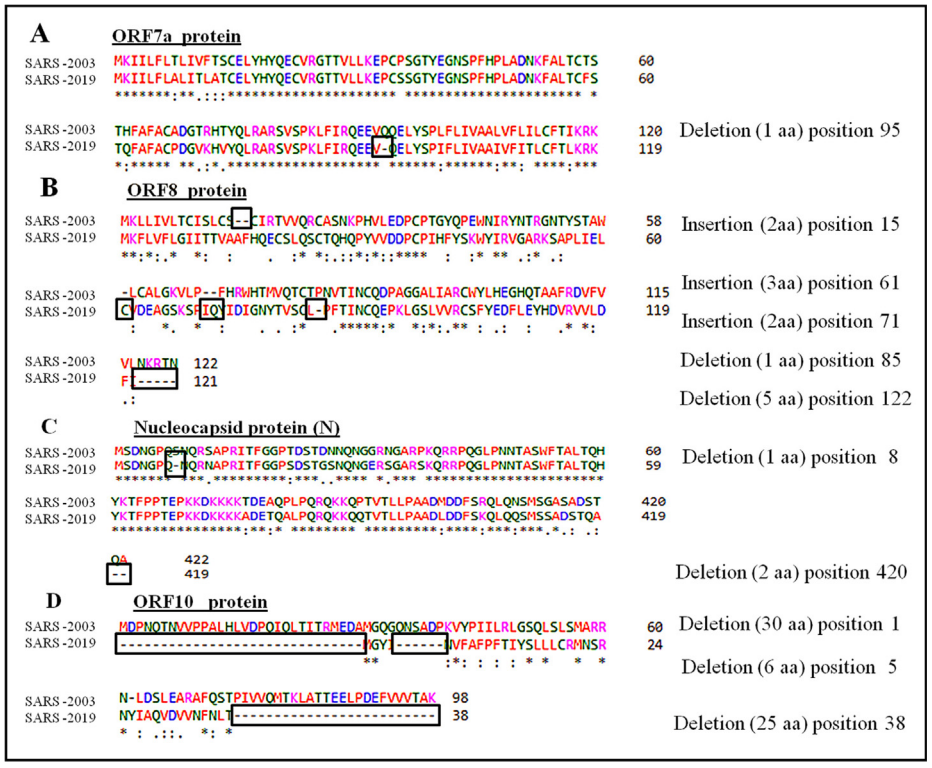

Fig. 7. CLUSTAL O (1.2.4) multiple proteins sequences alignment of SARS-CoV-2 (SARS-2) and SARS-COV (SARS-1) proteome. (A) Alignment of the variable regions of ORF7a protein. (B) Alignment of the variable regions of ORF8 protein. (C) Alignment of the variable regions of nucleocapsid protein. (D) Alignment of the variable regions of ORF10 protein. Del: Deletion, Ins: Insertion.

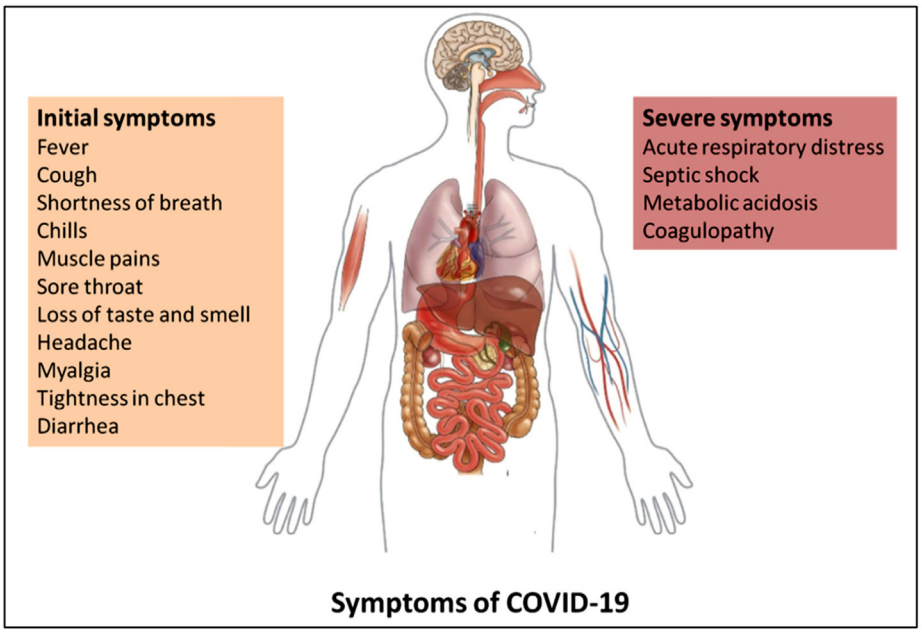

Fig. 8. Initial and severe clinical symptoms of COVID-19 (Control and Prevention, 2020).

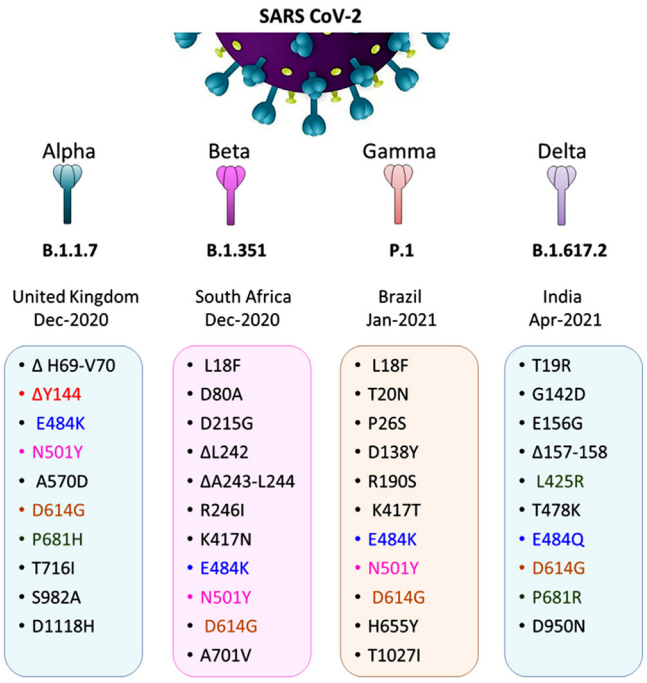

Fig. 9. SARS-2 variants and their essential mutations within the spike protein. The variants are referred as alpha, beta, gamma and delta based on WHO labeling system and classified into lineages according to Pang nomenclature. Country and time where and when the variants were firstly characterized are shown. The viral variants as well as their characterizing mutations are color coded.

10. SARS-2 variants of concern (VOC)

10.1. Alphacoronavirus (alphaCoV)

The alphaCoV was emerged in by the end of 2020 in the United kingdom, and identified as a new variant based on the sequencing of SARS-2 genome from positive tested patients(Galloway et al., 2021; Volz and Mishra, 2021). The alphaCoV variant contains 17 mutations distributed throughout the genome, in comparison with the parental SARS-2. Eight of which are present in the S protein that enhanced the ability for attachment and entry of host cells (Davies and Abbott, 2021; Walensky et al., 2021; Wu et al., 2021). Patients infected with alphaCoV variant showed an increased intensity of the disease compared to people infected with other forms of SARS-2 variants (Davies et al., 2021; Volz and Mishra, 2021). Clinical reports of patients in the UK indicated that the death risk ratio of individuals infected with alphaCoV was 1.64 higher than those infected with previously circulating strains of the virus (Challen and Brooks-Pollock, 2021). The alphaCoV variant emerged as one of the most dominant SARS-2 variants in the USA.

10.2. Betacoronavirus (betaCoV)

Another variant of SARS-2 is betaCoV generated in the second wave of coronavirus disease, emerged in South Africa in October 2020 (Tegally et al., 2021). This variant includes nine mutations in the S protein; three of which enhance the binding affinity to the ACE2receptor (Mwenda et al., 2021; Wibmer et al., 2021; Wu et al., 2021). The betaCoV variant was recorded in the USA in January 2021. This variant has a higher ability for transmission and decreased neutralization by antibodies (Wang et al., 2021a).

10.3. Gammacoronavirus (gammaCoV)

The third variant of concern, gammaCoV, was recorded by the end of 2020 in Brazil and identified in USA in January 2021 (Faria et al., 2021). The gammaCoV variant harboring 10 different mutations in the spike protein too. It also harbors three mutations similar to the betaCoV variant (Faria et al., 2021). The gammaCoV variant has decreased neutralization by antibodies therapy (Wang et al., 2021a).

10.4. Delta coronavirus (deltaCoV)

The recently emerged variant is deltaCoV, which was initially detected by the end of 2020 in India. This variant was the force behind the deadly second wave of COVID-19 that appeared in April

Table 6
Most studied amino acid mutations that characterize SARS-2 variants and their potential roles in viral fitness.

Mutant	Description	Role
L18F	Leucine-to-phenylalanine	Reduce neutralization by some antibodies.
ΔH69–70	Histidine deletion	Change the conformation of an exposed NTD loop and associated with increasing infectivity.
ΔY144	Tyrosine deletion	Seem to change the conformation of the N3 NTD loop (amino acid positions 140–156) and reduces antibody binding affinity.
K417N	Lysine-to- asparagine	Reduces virus sensitivity to antibodies and increases binding affinity to human ACE2 receptor.
N439K	Asparagine-to-lysine	Increase the binding affinity for the ACE2 receptor and reduces the neutralizing activity.
L452R	Leucine-to-arginine	Confer stronger affinity of the S protein for the ACE2 receptor.
T478K	Threonine-to-lysine	May enhances viral virulence as it was found in the rapidly rising SARS in Mexico and South America.
E484K	Glutamic acid-to-lysine	An escape mutation: immune dominant spike protein residue with various substitutions; facilitating escape from several mAbs and evade the immune system.
E484Q	Glutamic acid-to-glutamine	May be functionally similar to the antibody-escape mutation E484K.
N501Y	Asparagine to tyrosine	Increases in human ACE2 binding affinity conferred by a single RBD mutation.
D614G	Aspartic acid-to-glycine	Found in highly transmissible lineages like B.1.1.7, B.1.351 and P.1. it reduces the S1 shedding and increases the infectivity.
P681R	Proline-to-arginine	Boost cell-level infectivity of the variant and thus helps virus entry and abolish phospho-inhibition at S1/S2 site.
P681H	Proline-to-histidine	May increase spike cleavage by furin-like proteases.
D950N	Aspartic acid-to- asparagine	May participate in the regulation of s protein dynamics.

2021in the same country. DeltaCoV was rapidly diffused worldwide, and detected in USA in March 2021. DeltaCoV variant has 10 mutations in the spike S protein, as well. The scientific community predicted the deltaCoV variant to be the most dominant SARS-2 strain in USA in the next few weeks (Wang et al., 2021a).

11. Approaches for COVID-19 therapy

11.1. Traditional approaches

11.1.1. Killed or attenuated virus as a vaccine:

Coinciding With the emergence of a new pathogen, a quick and simple solution is urgently needed to develop a vaccine against this disease. Therefore, it makes sense that the traditional approach (Fig. 10). Using inactivated or attenuated viruses based on cell culture would be the fastest and easiest way to develop a coronavirus vaccine, as previously made with many commercial inactivated vaccines against many viral diseases.

Killed or attenuated virus vaccines depend on maintaining the spike S protein or the whole attenuated SARS-CoV (Roberts et al., 2008). This approach enables potently elicit high levels of antibodies in animal models (Tsunetsugu-Yokota et al., 2006). In this approach, the viral inoculum is exposed to certain chemicals such as formalin or physical forces such as gamma-ray to attenuate the virus viability so it doesn't cause disease, but still capable of triggering the host immune system. Using this approach, a commercial Beta-propiolactone Sinopharm, and Sinovac attenuated COVID-19 vaccines we reproduced in China. For a long time of successful applications, of polio and smallpox vaccines, inactivated vaccines promote the immune system similarly to natural infections

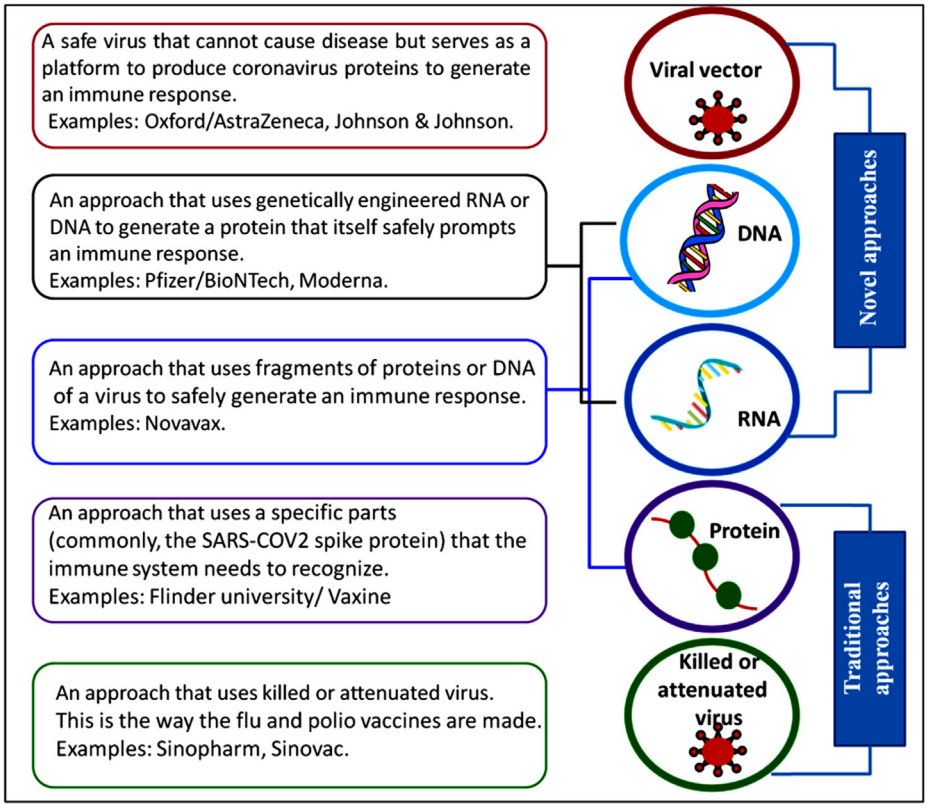

Fig. 10. Traditional and novel approaches of COVID-19 vaccines production.

generating natural viral antigens over a long period of time (Enjuanes et al., 2016; Roberts et al., 2008). With the emergence of MERS, a gamma-ray attenuated vaccine was developed to face the spreading of pathogen. This vaccine induced high levels of antibodies but caused lung pathological changes in vaccinated animals (Bolles et al., 2011; Tsunetsugu-Yokota et al., 2007). Similarly, attenuated MERS vaccines appear to trigger hypersensitive-type lung pathology risk similar to that found with attenuated SARS-1 vaccine(Agrawal et al., 2016).

11.1.2. Protein-based vaccine

Protein-based vaccines are composed of peptide subunits derived from targeted viruses(Hansson et al., 2000). Conversely with conventional vaccines, protein-based vaccines have fewer side effects and higher safety. However, it is not clear whether the immune response will be correctly initiated. Therefore, ingredients and vaccine delivery systems are important to promote immunological response (García and De Sanctis, 2014).

The majority of coronavirus vaccines focus on the S protein, which is responsible for ACE2 receptor binding via its receptor binding domain (RBD)(Choi et al., 2017; Okba et al., 2017). Recently, *Pichia pastoris* yeasts were used to produce a huge number of modified peptides in the culture medium without the need of animal-derived growth factors, thus largely applied in the industries of vaccines. Further investigations on both of the S and N viral proteins and their specific antibodies are essential.

11.2. Novel approaches

11.2.1. RNA and DNA-based vaccines

RNA and DNA-based vaccines consisting only of DNA or RNA (Fig. 10). Such type of vaccine is getting injected to host cells to be translated into a specific protein to induce immunological responses. Naked RNA and DNA molecules are not generally a subject to preexisting immunity that can disserve the efficacy of recombinant virus vaccine due to the lack of viral coat. Nucleic acid vaccine is safer and has low production costs; providing some advantages compared to other approaches. Reproduction of post-translational modifications of the plasmid encoded protein, maintaining vaccine immunogenicity and cellular immune-promoting abilities, at the same time (Liu, 2011; Sardesai and Weiner, 2011). Researches proven that the integration of the genes of the virus into host genes using vectors is extremely rare(Sardesai and Weiner, 2011).

Since the first coronavirus pandemic in China, SARS-1vaccine was developed based on the spike (S) coding DNA sequence that gets translated into detectable amount of protein sufficient to trigger IgG antibody levels and CD4 + and CD8 + T cell responses (Huang et al., 2006; ZhaoP, 2004). In addition, significant elevation of the S protein-specific IgG1 and IgA in the respiratory tracts of mice was also detected.

After the emergence of epidemic MERS in 2012, there were several developed nucleic acid vaccines namely pVax1™ (GLS-5300), pVRC8400, and pcDNA3.1-S1 encoding for MERS-CoV S1 subunit (Chi et al., 2017; Muthumani et al., 2015; Wang et al., 2015). These vaccines acted to induce and neutralize antibodies and immunological responses in animals like monkey, camel, and mouse. The antibody IgG level of S1 subunit of MERS-CoV was higher than the antibody of the complete S protein. Additionally, mixing of MERS whole S protein vaccine with enhanced S1 subunit generates antibodies and reduced disease severity in monkeys. Compared with single protein vaccines, the combination of nucleic acid and protein led to more efficient vaccination and stronger immune response (Wang et al., 2015).

Regarding SARS-2, a respectable numbers of vaccine development projects initiated after the emergence of this virus, especially RNA and DNA-based vaccines. The use of nucleic acids vaccines is innovative and relatively safe, particularly the mRNA-based vaccine. As it is artificially synthesized, the development of the product will be much faster. RNA based vaccines against SARS-2 developed by Moderna and BioNtech/Pfizer have entered clinical application.

11.2.2. Viral vector vaccines

Virus-based vector vaccine can efficiently introduce gene/genes encoding viral antigen into patients. The injected patients produce antigen within a certain period after vaccination (Enjuanes et al., 2016). Multiple injections are required to promote the systemic immunological responses due to subunit vaccine and protein-induced immune response is usually short-lived. In contrast, non-attenuated viral vector can naturally invade the cell, thus induce stronger immune responses. Several viral vectors for coronavirus vaccines have been developed(Schindewolf and Menachery, 2019). These viral vectors provide promising directions for coronavirus vaccine research and development. There are several replicating and non-replicating viral vector vaccines under development for COVID-19. As an example of this approach, AstraZeneca as a virus-based vaccine was developed based on a single recombinant, replication-deficient chimpanzee adenovirus vector encoding the spike protein of SARS-2. This novel vaccine is now approved and available for commercial applications.

12. Common questions

12.1. What is the protection duration that coronavirus vaccines provide?

It's still early to determine the protection longevity of coronavirus vaccines, as the vaccines have been recently developed. Investigations are currently ongoing to answer this question. However, it seems that the current data suggest that convalescent's patients develop immunological responses that provide at least some period of protection against reinfection. Although, the strength and longevity of this protection are yet to be elucidated.

Lan et al. (2018) published the very quickly dropping of antibody titer in some recovered patients, which suggested that they may get reinfected by SARS-2. In the same context, it is reported that some patients were diagnosed to have SARS-2 reinfections within three months of their first infection (Liu, 2011). The same results were later published by other researchers (Huang et al., 2004). Reinfection suggests that the immunity against coronavirus may decline rapidly, or the virus evolution rate is apparently quick.

12.2. How quickly cancoronavirus vaccines stop the pandemic?

Several factors affect the vaccines impact on the pandemic of coronavirus such as the effectiveness of the vaccines, the probability of evolving novel variants, the human genetic makeup and the number of vaccinated people within affected community. Scientific reports proved that coronavirus vaccines provide a high level of efficacy and the WHO is working to ensure that approved vaccines are effective, so they can have great impact on the pandemic.

12.3. Do other vaccines protect against coronavirus?

There are no evidences that other vaccines, apart from currently developed for the SARS-2 virus will sufficient to prevent coronavirus disease. This might be because of the specificity of the genetic responses to a certain vaccine. However, the scientific community found that some vaccines such as influenza, measles, pneumonia and polio vaccines can all offer some level of non-specific

protection (Ritz et al., 2013; Shann, 2013) against infection of SARS-CoV-2.

12.4. What are the benefits of getting vaccinated?

The production of coronavirus vaccines to protect against the disease was based on the developing of immunity responses to the SARS-2virus. Immune responses as a result of getting vaccine mean the reduction of risk developed by the illness and its consequences. These immune responses help the person to fight back against the possible virus infection. Vaccination may also provide the protection of other people whom could get infected particularly or people who have risks for severe illness from coronavirus, elderly adults, and people with other medical disorders.

12.5. Who should get vaccines?

According to WHO instructions, all coronavirus vaccines are safe for people older than 18 years, including patients with any conditions and auto-immune disorders. These conditions include hypertension, diabetes, asthma, pulmonary, liver and kidney disease, as well as chronic infections that are stable and controlled.

12.6. Can we stop taking precautions after being vaccinated?

Vaccines protect us from serious illness and dying from the virus. For the first two weeks after getting the vaccine, we do not have high levels of protection, and then it is gradually increases. Two doses of the vaccines must be taken to achieve the highest levels of immune response.

12.7. Are both shots should be the same type of vaccine?

Some trials are carried out to investigate whether we can have the first shot from one vaccine and a second one from a different vaccine. The available data about these trials aren't sufficient to recommend any combination.

12.8. Can the coronavirus vaccine cause a positive result for a PCR or antigen test?

No, the vaccines will not cause positive test results for the test checks. Because the coronavirus vaccines prompt the immune system, but it may cause positive test in serological assays that measure coronavirus infection in individuals.

12.9. Should the person be vaccinated if he has had coronavirus?

Even if you have already had coronavirus, you should get vaccinated at several weeks post infection. The protection that someone gains from getting coronavirus is not equal in all people, and its longevity isn't determined (see Tables 5 and 6).

12.10. Are the vaccines safe for children?

Normally the vaccines are tested in adults first, to keep children from probable risks. Now it becomes clear that the vaccines are safe for adults, and are being studied in children as well. Once those studies are done, the guidelines will be proclaimed. Concurrently, the children must be in physical distance from others, wash their hands frequently, sneeze and cough into their elbow and wear masks when possible.

12.11. Do the vaccines protect against variants?

The current vaccines of coronavirus are expected to give some protection against SARS-CoV-2 variants and prevent serious illness and death. When a vaccine becomes less effective against one or more mutants, the composition of the vaccine should be changed. Interestingly, we must do our best to stop the virus evolution and dissemination. This could be achieved by keeping social distance, covering up while coughing or sneezing, washing hands frequently, wearing authenticated mask and avoiding populated and poorly ventilated places.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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